Supporting Materials and Methods

Purification of Hop2 and Mnd1 Protein Complexes. cDNA clones were used as template DNA for sticky end PCR cloning (1). *E. coli* BL21-CodonPlus(DE3) host strain harboring both Hop2 and Mnd1-His-6 expression vectors were induced with isopropyl β-D-thiogalactoside (IPTG) for protein expression. Fifteen milliliters of overnight culture of *E. coli* BL21-CodonPlus(DE3) host strain harboring Hop2 and Mnd1-His-6 expression vectors was inoculated into 1 liter of LB broth with 30 μg/ml kanamycin, 50 μg/ml ampicillin, and 1% glucose at 37°C for ≈3.5 h (the time required to achieve an optical density of 0.6 at 600 nm). The cultures were cooled to 20°C in an ice bath, and the recombinant proteins were induced with 1 mM IPTG for 6 h at 20°C. Cells were harvested by centrifugation and resuspended in 25 ml of lysis buffer (20 mM Tris·HCl, pH 8.0/300 mM NaCl/5 mM β-mercaptoethanol/1 μg/ml leupeptin/0.1 mM L-1-tosylamido-2-phenylethyl chloromethyl ketone). Protein purification was carried out at 4°C. Bacterial cells were disrupted at 30,000 psi with a French press (Sim-Aminco, Rochester, NY). The lysates were centrifuged at 100,000 × *g* to separate soluble protein. Soluble lysates were loaded on a 5-ml Talon metal affinity column (Clontech) equilibrated with lysis buffer plus 10 mM imidazole. The column was exhaustively washed with the same buffer (≈30 column volumes) until the absorbance at 280 nm reached baseline. The column was further washed with a buffer containing 30 mM imidazole, and H2M1 complex was eluted from the column by using a buffer containing 150 mM imidazole. For enzymatic assays, H2M1 complexes were further purified by using a size-exclusion Superdex 200 HR10/30 column.

Gel Filtration and Sedimentation. The average elution position (K_{av}) in gel filtration was computed by using the equation $K_{av} = (V_e - V_o)/(V_t - V_o)$, where V_e and V_t represent elution volumes for the sample and smallest standard (i.e., tryptophan), respectively. V_o is the void volume, determined by the elution volume of Blue dextran 2000. Sedimentation coefficient (S) and Stoke's radius (a) were used to estimate molecular mass based on the following equation: $Mr = (6.02 \times 10^{23} \times 6\pi \times \eta_{H20} \times a \times S) / (1 - \nu \times \rho_{H20})$, in which the density of water (ρ_{H20}) is 0.99 g/cm^3, and typical partial specific volume (ν) of protein macromolecules is 0.68-0.72 cm^3/g (2). The viscosity coefficient of a protein solution is ≈1.12 g × sec/m.

DNA Strand Assimilation Assay. Dmc1 (1 μM) was preincubated with either ^{32}P-labeled oligo PA1655 or PA1656, or both (6 μM each) in the presence of 1 mM magnesium acetate and 2 mM adenosine 5'-[β,γ-imido]triphosphate (AMP-PNP). H2M1 proteins (0-16 μM) were also preincubated with either pUC18-Kan or GW1 or both (40 μM) in the presence of 20 mM magnesium acetate and 2 mM AMP-PNP. After 2 min at 37°C, equal volumes (10 μl) of both reaction mixtures were mixed together to initiate the DNA assimilation reactions for 15 min. Reactions were stopped and deproteinated by the addition of SDS and proteinase K to a final concentration of 0.25% and 0.25 mg/ml, respectively, and incubated at 37°C for 2 min. DNA from the reaction mixtures was then resolved on a 0.8% agarose gel in 1× Tris-acetate-EDTA buffer for 6 h at 4 V/cm. Gels were semidried with Whatman filter paper and directly analyzed by phosphoimaging. To estimate DNA assimilation efficiency, an aliquot (1 μl) of total reaction mixture was

taken, serially diluted, spotted onto Whatman filter paper, and quantified by phosphoimaging.

1. Shih, Y. P., Kung, W. M., Chen, J. C., Yeh, C. H., Wang, A. H., & Wang, T. F. (2002) *Protein Sci.* **11,** 1714-1719.

2. Cantor, C. R. & Schimmel, P. R. (1980) in *Biophysical Chemistry*, eds. Cantor, C. R. & Schimmel, P. R. (Freeman, San Francisco), Part II, pp. 539-590.